Organic Microchemistry

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MICROCHEMICAL analyses of organic compounds still follow largely the pattern set by Fritz Pregl. Considerable strides have been made during the past 6 years, as evidenced by the increase in the number of microchemical laboratories and the wider application of microanalytical chemical techniques. During the late twenties and early thirties, microanalytical methods were but little used in the United States except in the Atlantic Coast states. Ten years later these methods had been introduced into the midwestern states, and today micromethods are in use in analytical laboratories throughout the country.

The keen interest shown in the development of microchemical analysis is exhibited by the establishment of a committee on the performance of microchemical balances (A. H. Corwin of Johns Hopkins University, chairman); a revival of the committee on standardization of microchemical apparatus (A. Steyermark, chairman); and the re-establishment of a referee on the standardization of microchemical methods by the Association of Official Agricultural Chemists. The British Standards Institute has formed a subcommittee to standardize microchemical apparatus, and the work of this subcommittee is well advanced. Microchemistry has gone a long way since Pregl devised this scheme of analysis, for it is no longer limited to the analysis of material available in only small amounts. Instead, it has an accepted place, along with micromethods, for the analysis of materials available in large amounts. The micromethod is superior, in that it is faster, more economical of reagents, and in many instances more precise and accurate.

In expanding micromethods, some analysts have used larger samples and others extremely small ones. The committee on apparatus is now in the process of analyzing the results of a questionnaire by which it hopes to determine the extent to which semimicroapparatus is used and how much adherence there is to the microequipment. Because of the expansion of micromethods to cover weights from a few hundredths of a microgram to 50 mg., they have been forced to prefix the term micro with such descriptions as "semi" and "ultra." These prefixes are not adequate, because each may cover so wide a range; thus semimicro may mean anything from 10 to 50 mg. Even the term micro is being misused, for some analysts make a distinction between methods employing weights of 2 to 5 mg. and those of 5 to 10 mg. To avoid much of the ambiguity caused by a not too well-defined prefix, it might be desirable to drop all prefixes and use only the word micro followed by a numeral to show the top range of the weights involved.

Hallett (79) in his extensive review published in 1942 listed the basis for selection of certain weights and the accuracy that could be expected, and included detailed descriptions of most of the apparatus and methods of organic microanalysis available then. This review is therefore limited to papers published since 1942. It is somewhat surprising that during this period, which included World War II, considerable strides have been made in this country. Our supplies of apparatus, particularly microbalances, had been cut off, and it was only through the efforts of our American laboratory supply houses, apparatus, and balance manufacturers, and glass blowers that we had available the microchemical equipment required for the expansion of microchemical laboratories. For this an acknowledgment is due.

BALANCES

Few changes, if any, have been made in balances in the past few years, except for the development of the modern quartz microbalance. In 1942, Manigault and Tsai (153) developed such a balance with a magnetic compensator. Later in 1943 Nanjundayya and Ahmad (161) developed a quartz torsion balance suitable for loads up to 0.5 mg. with a sensitivity of 0.0025 mg. In 1944 Gulbransen (77) reported the construction of a quartz balance, to be operated in a vacuum, having a sensitivity of 0.3×10^{-6} gram with fairly large samples.

In 1947 Kirk, Craig, Gullberg, and Boyer (120) reported the development of a quartz balance which could handle loads up to 300 micrograms and had a sensitivity of at least 1 minute of arc per 0.005 microgram with loads of 0.1 to 0.2 gram. This ultramacrobalance is said to be more rapid and reproducible than the standard microchemical balance and free of vibration effects. These ultramicrobalances with high sensitivity have been used principally in inorganic chemistry.

In 1947 Stock and Fill (225) reported the development of an all-glass, oil-damped semimicrobalance. The balance did not have great accuracy, but it was rapid and filled a definite need. Mettler has developed a semiautomatic semimicrobalance of one-pan construction with a constant load (6). A counter automatically totals the weights removed to compensate for the object weight; the decimal weights are obtained by means of an illuminated projected scale. If it is like its counterpart, the macrobalance, one of the few objections will be that it cannot be used with a tare.

For the most part, balances of domestic manufacture in use in organic microanalysis are not air-damped. In Europe, nearly all microbalances appear to have an air-damping system, and on the Continent a multirider control is used in addition. The Bunge microbalance uses air damping beneath the pans, whereas the Sartorius uses overhead damping, for which it is claimed there is less chance of dust entering the air chamber (15). Although air-damped, the British Oertling balance does not use the multiple rider.

An American microbalance of new design, the first in some years, is now under test, and probably will be made available in the near future. An outstanding feature is the short-arm beam, which helped to make the Kuhlmann balance so popular.

Whether or not it will be possible to construct a fast and accurate balance built on electronic principles, either magnetic or piezo effect, is still speculative. Clark (39) has described one which employs a controlled magnetic lifting device that compensates for the sample load, making the instrument in a sense a null instrument. It is claimed that a load of 1 microgram can be measured with the balance.

A discussion of the accuracy and the reliability of various kinds of balances will not be attempted here, as such studies are now being made by the AMERICAN CHEMICAL SOCIETY's committee on microchemical balances. Martin (156) discussed the different types of balances, the qualities of a good balance, and possible errors. Corwin (43) in 1944 made a report on the errors of the microbalance, and described methods for locating and reducing the magnitude of these errors. In 1943 (191) the committee on microchemical balances published results which serve as a guide for measuring the performance of an analytical balance.

To increase the speed of the Kuhlmann balance, Tuttle and Brown (234) developed a device for projecting a magnified image of the pointer scale.

Wilson (257) has listed major factors to be considered as sources of error in microweighing, and has discussed the precautions necessary for cleaning the microbalance.

ELEMENTAL ANALYSIS

CARBON AND HYDROGEN

Micro- or macrodetermination of carbon and hydrogen by catalytic combustion has been a somewhat empirical procedure. In most determinations, it is possible either to reduce the number of variations that can occur and so take measures to ensure that they are controlled during the analysis or to test the end products or residues for completeness of recovery of materials. This cannot be done with the carbon and hydrogen microdetermination by catalytic combustion. The only alternative, after everything appears to be in proper working order, is to use a sample of known purity. If the results are right, the unknown sample is analyzed, and this is followed by another analysis of the known standard. Unfortunately, even this may not succeed; in many cases although the theoretical results for the carbon and hydrogen of the standard were obtained, the results for the carbon and hydrogen of the unknown were low. There can be many causes for this error. A principal one is that the standard material is not similar enough in structure and composition, both of which materially affect the completeness of the burning of the sample to carbon dioxide and water.

Although this determination is one of the oldest and most frequent of microchemical operations, when microchemists were questioned (253) as to the microchemical procedure that should be first investigated with the object of developing a standard method of analysis, a study of the carbon and hydrogen analysis was almost a unanimous choice. As in other microprocedures, there has been a definite trend on the part of many microanalysts to use semimicromethods where possible, with a hope for the elimination of many of the errors that are so easily encountered in microanalysis.

There is considerable variation of opinion as to combustion-tube packing, rate of oxygen flow, and temperature of the combustion. Belcher and Spooner (16) advocated high temperatures (800° C.) and a rapid flow of oxygen (500 ml. per minute), with silver wool to absorb the interfering acid gases. For combustion at 195° C. of compounds containing nitrogen in addition to halogens and sulfur, Ingram (105) has attributed errors to the inefficiency of granular lead peroxide. He believes that best results can be obtained if the filling consists of pumice impregnated with silver vanadate instead of copper oxide and lead chromate or platinum contacts. He reports good success at 300° C. with ceric oxide deposited on silver chromate. These low temperatures of combustion are little used in this country; however, there has been some controversy concerning the temperature for both volatilizing and burning the sample. Although many prefer to keep the temperature at 650° to 750° C. (40, 55, 83, 96, 219), there is a trend toward increasing it, even as high as 1000° C. (8, 232). The need for a preheater or preburner is also doubtful. It is likely that for standard analyses they will be eliminated, with the use of a constant blank for a given supply of oxygen.

It has been known for many years that during the combustion in oxygen nitrogenous compounds produce oxides of nitrogen. Heron (86) claims that practically all the nitrogen of nitro compounds exists as oxides after the combustion and that 1 to 40% of the nitrogen not attached to oxygen in the compound is converted to oxides. The incomplete removal of these oxides of nitrogen is a source of error in the carbon analysis. The customary method for their removal is to pass the products of combustion over lead peroxide maintained at constant temperature. Heron (87) found that lead peroxide used in tubes either as lead peroxide–asbestos or in the granular form gave high carbon values. To effect a better removal of the nitrogen oxides, a liquid scrubber was placed between the hydrogen (water) absorber and the carbon dioxide absorber. Heron (87) used chromic-sulfuric acid as the scrubbing liquid. The use of a liquid absorber for oxides of nitrogen in organic combustion analysis was suggested by Elving and McElroy (52) in 1941, and Horning (96) reported best re-

sults with it; yet it has not been generally accepted here. In Europe, Étienne (55) reported good results, and Belcher (15) found that many of the microchemical laboratories on the Continent were using the liquid absorber.

A dry absorber for the oxides of nitrogen, which unlike lead peroxide operates at room temperature, is Hopcalite. This was reported as early as 1937 (44) by Corwin, who is still using it in his carbon and hydrogen combustion trains. Hopcalite, however, has never been widely accepted; consequently, few have commented on it.

The automatic carbon and hydrogen combustion furnaces have been the subject of much controversy. Today they have been fairly well accepted. Clark and Stillson (40) and Horeischy and Bühler (95) have described semi- and fully automatic combustion apparatus, adding to the already extensive literature (40, 219). The rate at which the burning or vaporizing furnace should move has not yet been settled, and consequently the only commercially available automatic device is provided with a variable-speed control. Many analysts, especially those using less than 10-mg. samples, heat the packed portion of the tube with an electric furnace, but some still prefer to vaporize the sample with a gas heater. In this country, we have more or less accepted automatic burning for carbon and hydrogen analysis, but English analysts have not accepted it. German (15) laboratories have adopted it almost 100%. In one laboratory, they have gone so far as to develop an automatic chamois wiper for the absorption tubes.

No attempt is made here to discuss tube fillings. Although they are varied, they are all essentially similar to the original Pregl filling. It is hoped that the work on standardization of methods may lead to some uniformity. The size of the combustion tube is also undergoing considerable discussion. Although the committee on standardization of microchemical apparatus will recommend a size for use in microanalysis (up to 10 mg.), it has not received complete replies to inquiries regarding the size of the tube in general use. Among analysts who use semimicrosamples (20 mg.) or larger there is a definite trend toward using a tube of small inside diameter, one closely approximating that recommended for microanalysis.

For the regulation of gas flow, Furter and Steyermark (60) have described an apparatus which has an attached scale and uses no metal parts; the train uses the Mariotte bottle. Irimescu and Popescu (109) have proposed burning under positive pressure, with the combustion train attached directly to a reducing valve on a gas cylinder instead of a gasometer and pressure regulator. Goulden (74) uses an automatic regulator and manometer. It is possible that these systems will gain in acceptance, especially if the combustion is made with a definite volume of gas.

Several new absorption tubes have been proposed. MacNevin and Varner (152) have described the use of absorption tube tares without wiping, which permits the use of Pyrex tubes. Lacourt (129) has described a Friedrich type of absorber and has given methods of sealing. Langer (133) has developed a tube which eliminates the air jacket of Friedrich and the cap and sleeve of the Abrahamiczik tube.

Some compounds do not lend themselves readily to the catalytic analysis of carbon and hydrogen, particularly those of explosive nature and in some cases long-chain fatty acids. For these it is becoming a practice to determine the carbon only, using a wet combustion procedure. A simple method for 15- to 20-mg. samples is the adaptation of the Van Slyke manometric amino nitrogen apparatus, using chromic oxide, potassium periodate, and fuming sulfuric acid in phosphoric acid as oxidants, then measuring the volume of the carbon dioxide gas formed.

More recently, Houghton (99) suggested an apparatus for samples containing 2 to 5 mg. of carbon and an oxidizing solution of phosphoric, sulfuric, and chromic acids. The liberated carbon dioxide is trapped in a barium hydroxide solution, the excess of which is back-titrated with standard acid. Oberhauser (171) and

others have conducted the oxidation with potassium persulfate and silver sulfate, trapping the carbon dioxide in 30% potassium hydroxide, noting the increase in weight or in 0.5 N potassium hydroxide, and determining the carbon dioxide by Winkler's method.

NITROGEN

Determination of nitrogen has been the subject of a large number of papers in the microchemical as well as in the macrochemical field. The object is to devise a simpler and more rapid but accurate method. Because of the simplicity of the apparatus and other desirable features, the Kjeldahl method has been well received. Its use in the determination of nitrogen has been largely limited to naturally occurring mixtures, principally in the field of biochemistry (9). For the analysis of pure nitrogenous compounds, this method has been more restricted, and has been accepted with some skepticism, as it has been demonstrated time and again that nitrogen cannot always be completely recovered from certain refractory types of compounds, chiefly those containing heterocyclic nitrogen.

The controversy over the ideal combination of catalysts of the past decade is still going on. Beatty (11) has advocated a large amount of crystalline anhydrous copper sulfate, the undissolved crystals serving as boiling chips, and has advised the addition of selenium for semimicrosamples. Sobel, Mayer, and Gottfried (211), in their adaptation of the Kjeldahl method for urea and other nitrogenous compounds, use the same flask for digestion and distillation and are able to determine total nitrogen in as little as 0.01 ml. of blood. Kaye and Weiner (112) have modified the method of Clark (38) by using the mixed indicator of Ma and Zuazaga (149), which consists of bromocresol green and methyl red. Others (172) have since varied the ratio of the indicator to the dye in this mixture. Kaye and Weiner (112) advocate overtitration of the trapped ammonium solution with strong standard acid and back-titrating with a dilute alkali. Cole and Parks (42) have confirmed the observations of others that copper as a catalyst used in the absence of selenium and mercury gives low results, while mercury and selenium oxychloride catalyst give excellent recoveries on all nitrogenous compounds except those containing a nitrogen-to-oxygen or a nitrogen-to-nitrogen linkage.

Portner (183) has outlined a simple oxidation mixture which omits a metal catalyst. He uses bromine and 30% hydrogen peroxide in the presence of sulfuric acid. Care must be exercised, however, to prevent overheating and consequent carbonization. There is a definite trend toward the omission of all metallic catalysts except mercury, with a more exact control of the amount of salts and acids to be used in the digestion mixture. White and Secor (248) have shown that with this mixture the nitrogen can be quantitatively recovered from tryptophan and gramicidin without a hydriodic acid pretreatment. Ballantine and Gregg (9) use only phosphoric acid, sulfuric acid, and solid potassium persulfate in the digestion and have reported excellent results if the mixture is kept almost anhydrous to prevent the solution of the persulfate. They have also adapted the use of biiodate in the iodometric titration of the liberated ammonia. Brüel et al. (26) have given excellent directions for conducting micro-Kjeldahls. In addition to the complete description of microapparatus, they ve discussed catalysts, and although they were forced to use selenium because of the conditions of their experiments, they indicate that they were never able to account for more than 70% of the nitrogen of a refractory material like pyridinium-zinc chloride ($C_5H_5NHCl)_2(ZnCl_2)$. The proper conditions for the digestion are still uncertain, as pointed out by Kirk (119), who believes that enough attention has not been given to the salt ncentration, the time of digestion, and the control of the selenium as a catalyst. For use in aeration microtechnique, Sobel et al. (210) have described two new types of absorption tubes. One is a sealed-in bubble tube applicable to 10 to 200 mg. of ammonia; the other is an adaptaton of a conical centrifuge tube usable in the determination of 1 to 10 mg. of ammonia.

DUMAS NITROGEN

The Dumas procedure is perhaps still the most generally accepted method for determining nitrogen in all types of nitrogenous organic compounds. Few modifications in the procedure have been proposed in the past few years. Instead, attention is centered on improvement of the apparatus, both the combustion tube and the nitrometer.

Zimmermann (269) designed an apparatus in which he was able to complete an analysis of materials such as atropine satisfactorily in 25 minutes. With his apparatus the combustion takes place automatically. Copies of his mechanized Dumas apparatus are to be found in many of the German microchemical laboratories. He used solid carbon dioxide as the source of the inert gas, a practice which is becoming more and more popular, both here and abroad. The source and method of manufacture of this material are often of great importance. In the author's laboratory, it was observed that when the solid carbon dioxide was obtained from fermentation, the amount of nitrogen trapped with it made it utterly unsuitable for use in the Dumas method. Korshun and Hel'man (127) have described the apparatus using the Kipp generator, an electrical heater for the long burner, and a gas sample burner. Evidence that the minimum temperature for heating the sample tube is 650° C., with low and erratic recoveries of nitrogen below this temperature, has been presented recently in unpublished reports of Association of Official Agricultural Chemists referees.

Weygand (246) called attention to the fact that chance introduction of foreign material, such as that from rubber connectors, into the apparatus should be guarded against, and suggested an apparatus with glass-joint connections.

Gonick et al. (66) proposed an all-glass improved Dumas apparatus operated at 750° C. To ensure the complete combustion of refractory materials, the products of combustion and oxygen are recycled through the combustion tube until constant residual volume is obtained.

Kirsten (121) has proposed one of the first truly radical changes in the Dumas method. The temperature of the combustion is not sufficiently high for all types of compounds, but with copper filling temperatures much above 800° C. cannot be used. Kirsten, using a quartz tube with ground joints, suggests as the permanent filling nickel–nickel oxide, which allows the use of temperatures of 1000° C. and with backward stream washing results in a saving of time and longer life of the filling. The temporary filling of copper oxide is held in a quartz capsule.

To prevent possible adherence of gas bubbles on the surface of the mercury in the nitrometer, Pomatti (182) uses a small curved piece of iron wire, which is floated on the mercury and moved over the surface by means of an external magnet to sweep off any adhering gas bubbles. Müller (160) carried Weygand's idea one step further by developing a new azotometer, which eliminates rubber leveling tubes or connectors. The nitrometer is a compact instrument with the alkali chamber, gas buret, and leveling device all interconnected by glass. To avoid the most common sources of error of the nitrometer, Stehr (216) has replaced the conventional stopcock with a ball and socket valve, which offers many advantages. To eliminate much of the difficulty encountered with the precision stopcock for controlling the flow of carbon dioxide from combustion tube to nitrometer, Hershberg (88) devised a needle valve with glass seat and mercury packing. A new valve made entirely of stainless steel, which is rugged yet sufficiently light, has now been designed.

A great deal of difficulty has been experienced with the limestone-Kipp generator as a source of nitrogen-free carbon dioxide, especially during the war years, when a supply of satisfactory marble was nonexistent. Pagel (175) has designed a generator which uses pure bicarbonate as the source of carbon dioxide. The

apparatus supplies a good nitrogen-free carbon dioxide gas, but it is fragile and difficult to change.

Determination of nitrogen existing as nitrates is still a serious problem, partially solved by the adaptation of the Devarda method by Kieselbach (116) for determining 0.05 milliequivalent of nitrates. Leithe (137) has suggested an indirect method whereby the nitrate oxidizes ferrous iron of known titer, and excess of ferrous iron is measured with standard dichromate. Genevois (63) has outlined the formation of various nitro compounds, many of which can be analyzed by the titanium trichloride reaction.

HALOGENS

Determining halogens constitutes one of the major tasks of microchemists, as such analysis is important from the standpoint of identifying organic compounds and indicating their purity. In many compounds, determination of halogens is a fairly simple matter, especially if they exist in an ionic form. For the more refractory materials, the catalytic oxidation method of Pregl is still much in vogue, but many compounds are resistant to this treatment. As a result, many still prefer the Parr peroxide bomb method or the Carius method, which are generally known and so are not discussed here. Both methods, however, introduce such a high concentration of salts and other interfering materials into the solution that subsequent titrimetric procedures become difficult.

Elving and Ligett (51) proposed a method for decomposing fluorides and other halogen organic compounds by heating them with an alkali metal in an evacuated tube at moderate temperatures, forming the alkali metal halide, which can then be determined by conventional means. This method was described by Zimmermann in 1928 for the destruction of sulfur compounds, and also by Bürger (27). The potentiometric identification of the end point of the halogen titration [for which Dean and Hawley (47) and Northrop (170) have described electrical setups] appears to be a definite advance.

Irimescu and Chirnoaga (107) have adapted the method of Stepanov to microprocedure. This consists of converting the halogen of the organic compound to the sodium salt by heating it with a mixture of absolute ethyl alcohol and metallic sodium. Sisido and Yagi (206) digest the sample containing about 2 mg. of chlorine with an excess of sodium in butyl or amyl alcohol and titrate the resulting chloride ion with standard silver nitrate, with phenol blue as the adsorption indicator. Jurecek (110) decomposes the organic halogen compound by catalytic oxidation and passes the gaseous products over hot sodium carbonate, which picks up the acidic gases of bromine and chlorine to form the sodium salt. Iodine gases are trapped by heated silver dispersed on magnesium oxide. Peel, Clark, and Wagner (177) prefer the Parr peroxide bomb for decomposition and formation of the alkali salt. They strongly disapprove of the volumetric Volhard method for iodine and bromine analysis, because the end point is not certain except with too strong reagents. For liquid samples, they have proposed the use of gelatin capsules in the bomb. Christensen (35) has developed a micromethod for organic iodine based on its oxidation to iodate with permanganate. The iodate is measured in terms of liberated iodine with standard thiosulfate.

Harlay (80) has proposed a quantitative method for determining iodine which uses fluorescein not as an indicator but as a reagent. This is based on the fact that the diiodo derivative is nonfluorescent in ultraviolet light and its formation is controlled by the pH of the solution. By measurement of the decrease in fluorescence of the reagent, the amount of halogen present can be determined. The sensitivity of the test can be increased sixfold by converting the iodine to iodate and then liberating iodine from potassium iodide, thus increasing the amount of iodine six times over the original quantity. Dubouloz et al. (48) uses thiofluorescein, the synthesis of which is given as a colorimetric reagent for iodine. One atom of iodine in acid solution and two atoms in alkaline solution oxidize it to a colorless product. Its absorption curve shows a maximum at 5850 Å.

Shahrokh (205) has modified the chlorate digestion method fo determining iodine in biological materials so that larger sampl weights may be taken and the interference of iron and manganun eliminated. Teston and McKenna (232) use a high-temperature oxidation (1000° C.) for the destruction of halocarbon and are able to determine fluorine, chlorine, and carbon simultaneously. Small quantities of chloride ions can be determined readily by the method of Leithe (138), in which the chloride ion reacts with silver chromate to yield silver chloride and a soluble chromate. The chromate is easily titrated with dilute ferrous sulfate solution.

OXYGEN

A microchemical method which permits the direct determination of oxygen in organic compounds is perhaps one of the greatest advances in analytical chemistry for many years. It is now past the experimental stage, and its use will be demanded along with all other accepted methods for ultimate analysis as soon as there are definite indications that satisfactory equipment can be furnished. The high temperatures require a furnace that is not so easy to build as those for carbon and hydrogen, sulfur, and the halogens, and it may require some time before these furnaces become available.

Elving and Ligett (50) have presented a historical review of the methods used for the determination of oxygen, with special emphasis on the hydrogenation method of Meulen.

The method that has won great popularity for the direct determination of oxygen is the one first developed by Schütze (202) and later modified by Zimmermann (271) and Unterzaucher (235). It is the latter's modification that has been developed in this country by Aluise et al. (5). The essential features of this determination consist in decomposition of the sample in a stream of nitrogen made oxygen-free by passage over heated copper and the ultimate conversion of all the oxygen of the organic compound to carbon monoxide by passing the gases over heated charcoal which must be maintained at 1100° C. The amount of oxygen is then determined from the carbon monoxide, after it is converted to carbon dioxide with iodine pentoxide. The carbon dioxide is measured titrimetrically in terms of the liberated iodine or gravimetrically after absorption in Ascarite.

Zimmermann (271) uses an automatic burning furnace. The carbon monoxide is oxidized quantitatively at room temperature by means of a special preparation of iodine pentoxide. Unterzaucher prefers to oxidize the carbon monoxide with iodine pentoxide heated in an acetic acid bath and determine the liberated iodine titrimetrically. If the amount of iodine liberated is low, it is oxidized to iodate and thereby amplifies the titer. The nitrogen is purified at room temperature by using copper which has been reduced with hydrogen just before use. Aluise has described the construction of the furnaces for the destruction of the organic sample and for the conversion of carbon monoxide to dioxide. Details are given concerning the selection of the carbon and the iodine pentoxide. Somewhat less accuracy and precision have been obtained than those now obtained for carbon and hydrogen analyses. The presence of sulfur, nitrogen, and halogens does not interfere, and there is no problem of catalyst poisoning. Walton et al. (241) have given complete details the construction of the glass apparatus and the furnaces. They have found helium superior to nitrogen for providing the inert atmosphere of the combustion. For small amounts of oxygen. the carbon monoxide formed is measured colorimetrically with indication gel. Korshun and Hel'man (126) have also described a method similar to that of Unterzaucher.

Spooner (215) has attempted to avoid the high temperature 1200° C. required to reduce carbon dioxide to the monoxide with carbon by activating the carbon with sodium oxide. He was successful in doing this at 800° C. for one determination, but the

carbon deposited from the sample on the activated carbon caused it to cease to function.

SULFUR

In the past few years many have reported a "good" sulfur method. Zimmermann (268) developed a method based on that of Bürger. Potassium sulfide is formed by decomposing the organic sulfur compound with liquefied potassium. The excess potassium is destroyed with methanol, and the sulfide is converted first to hydrogen sulfide and then to sulfur. Later he trapped the hydrogen sulfide in cadmium acetate and determined the sulfur iodometrically (270).

Klein (122) has developed a colorimetric method for determining sulfur after it has been precipitated as benzidine sulfate. The precipitate is dissolved, diazotized, and coupled with Britton and Marshall's reagent to give a dark purple color, which can be measured in a photoelectric colorimeter. The method is said to be accurate to 0.05 to 0.150 mg. of sulfur.

To convert all the sulfur to sulfur trioxide, Ingram (104) conducts the catalytic oxidation of the sample in a stream of oxygen saturated with water vapor. After a neutral solution of basic mercuric cyanide is added, the sulfur products of the combustion can be determined titrimetrically with standard barium chloride. Any barium chloride in excess of the sulfate will react with the basic mercury salt, liberating hydroxyl ions which can be shown with an acid-base indicator. Korshun and Hel'man (128) decompose the substance in a stream of hydrogen over a glowing platinum catalyst. The sulfur is determined iodometrically after the hydrogen sulfide is absorbed in a zinc sulfate–acetic acid solution.

Irimescu and Chirnoaga (108) also decompose the sample in an atmosphere of hydrogen, but their contact catalyst is finely divided platinum deposited on small quartz tubes. They point out that a temperature of 1000° to 1100° C. is required for the conversion of sulfur of organic components to hydrogen sulfide. The catalyst becomes inactive as carbon deposits on it. The sulfur is determined titrimetrically. After the gas is absorbed in a silver nitrate solution and the silver sulfide precipitated, the excess silver is determined by Volhard's method.

Yagi and Egami (265) decompose the sulfur sample in a sealed tube containing fuming nitric acid and sodium chloride (Carius) and then determine the soluble sulfate by the method of Hinman. Horeischy and Bühler (94) also use the quartz bomb and nitric acid for decomposition, but determine the sulfur in the reaction products as sulfuric acid by evaporating on an evaporating-block at 105° to 110° C. to remove all other acids and titrating with standard potassium hydroxide. Sundberg and Royer (231) have described the decomposition of sulfur compounds and the collection of the soluble sulfate with a Grote combustion apparatus. The sulfur is determined titrimetrically with standard barium chloride, dipotassium rhodizonate being used as indicator.

In addition to a scheme of decomposition of organic compounds for sulfur, Alicino (1) has proposed a procedure for the identification of the dipotassium rhodizonate end point with standard barium chloride.

Ogg, Willits, and Cooper (174) have described a titrimetric technique for identification of the end point in the volumetric determination of soluble sulfates with barium chloride and dipotassium rhodizonate. They have eliminated one of the principal sources of error in using this indicator and have suggested means of avoiding a correction factor when small amounts of sulfur are determined as sulfates. Steyermark, Bass, and Littman (220) have applied the titrimetric technique of Ogg et al. to the analysis of organic compounds decomposed by the Carius method.

Wagner and Miles (239) have described a method using the Burgess–Parr oxygen bomb. The resulting sulfuric acid is determined with standard alkali after the sulfur is first precipitated and separated as benzidine sulfate.

Freri (58) has found that when the sulfur is determined gravimetrically as barium sulfate, the addition of 10% ethyl or amyl alcohol to the aqueous solution in which the precipitation is made facilitates the subsequent handling of the barium sulfate. As the precipitate does not lump, a simple spray or jet of wash water may be used.

Krocker filters the precipitated barium sulfate with a sintered-glass crucible (15). The solution is decanted, and the precipitate is washed directly from the precipitating dish to the filter according to macrotechniques. Although this is a rather startling procedure, less loss of sulfate is reported than when elaborate precautions are taken by using filter sticks, filter beakers, and the like.

GROUP ANALYSIS

ALKOXYL

Many workers have made suggestions for modification of the method for determination of alkoxyl to overcome erratic results and operational difficulties, in order that its usefulness can be extended to a wider variety of compounds and greater accuracy can be obtained with a simple rugged apparatus. White (249), using the method of Viebock and Brecher, redesigned the apparatus of Zacherl and Krainick (267) for the semimicrodetermination of both alkoxyl and halogens. Saccardi (192) modified the Zeisel apparatus to prevent bumping or surging of the hydriodic reaction mixture by leading the carbon dioxide through a capillary heated at 200° C. The hot gas is sufficient to keep the reaction mixture boiling smoothly. White (247) has discussed the effect and use of washers for scrubbing the distilled vapors of the alkoxyl analysis. Owing to the solubility of methyl iodide and its subsequent reaction with thiosulfate, thiosulfate used alone gave low values for methoxy- and methylimide. Ethoxyl- and ethylimide are not subject to this loss. This difference furnishes a simple means for rapid distinction between ethoxyl and methoxyl, provided a good washer can also be used. White has shown that the solubility of methyl iodide can be minimized by dissolving the thiosulfate in saturated sodium chloride or by adding cadmium sulfate to the thiosulfate solution. He has also shown that other wash waters containing phosphorus suspensions or 0.5% sodium carbonate are satisfactory.

Houghton (100) designed an apparatus, a modification of Clark's (37), which can be left unattended and still give reliable results. A rapid stream of carbon dioxide is introduced at the bottom of the reaction flask, and the sample spoon is so placed as to serve as a boiling chip. These, plus a rapid boiling rate, prevent bumping. To prevent distillation of the hydriodic acid, a water jacket is mounted on the tube. The trap holding the scrubbing liquid is so designed that the spent liquid can be easily removed and the trap refilled. An analysis is said to be completed every 20 minutes. In an effort to separate or identify the alkoxyl as methyl or ethyl, Houghton (98) substituted capillary U-tube immersed in acetone–solid carbon dioxide for the spiral trap of the previous apparatus. Nitrogen is substituted for carbon dioxide, and the alkali halides are condensed in the U-tube. The alkyl can be identified by density measurement accurate to 1%, but this requires a minimum of 30 mg. of sample. Boiling point determinations require only 3 mg. of sample, with a possible 5% error.

In the analysis of acetals and easily volatilized alcohols, low ethoxy values are often encountered. Hoffman and Wolfrom (93) attributed this to the loss of these volatiles from the reaction mixture after saponification, before they had reacted with the hydriodic acid. To avoid this loss, they designed a sample holder, for both liquids and solids, in which the entire sample is in contact with the reaction mixture long enough for the formation of the alkyl halide. To aid in the complete solution of the material during alkoxyl analysis, the solids sample holder or cup is sealed with a film of tartaric acid, making it possible to introduce the sample below the surface of the reaction mixture. They have

designed a new type of receiver that does not require a spiral for washing the alkyl halide from the gas stream. The authors claim good results.

Steyermark (218) also calls attention to the low alkoxyl values obtained with Pregl's apparatus for the analysis of volatile compounds, esters which split off the alcohol on immediate contact with hydriodic acid, or compounds with more than one methoxyl group. To avoid this difficulty, he has constructed an all-glass apparatus combining the essential features of the Fürter apparatus, which employs double distillation and is used successfully for the analysis of either alkoxyl or alkimide groups in volatile substances, and the Elek apparatus, which utilizes a water-cooled reflux condenser and a more efficient absorption tube for the alkyl halide. The reaction mixture is allowed to stand for some time before heating. Preparation of the hydriodic acid is of special interest. Steyermark is one of the first to suggest that a colorless acid of constant boiling point is unnecessary. His reagent is prepared by merely refluxing (air condenser) ordinary reagent grades of hydriodic acid for about 2 hours while a stream of carbon dioxide or nitrogen is bubbled through. The color of the resulting acid is not important, for even dark grades give no blank.

ACETYL

Clarke and Christensen (41) have supplied a simple procedure for determining acetyl groups in acetates of sugars and glucosides. The sample is placed in an Erlenmeyer reaction flask with a few milliliters of ethyl alcohol and a known volume (4 ml.) of 0.045 N sodium hydroxide. The mixture is shaken for 4 to 24 hours, and the excess of alkali is titrated with 0.05 N sulfuric acid to a phenolphthalein end point.

An improvement of the Kuhn-Roth apparatus for determining acyl groups has been made by Wiesenberger (251, 252). By redesign of the condensers and shape of the reaction vessel, it is possible to obtain in a few minutes a distillate free of high-boiling acid, and the hydrolysis can be accomplished with phosphoric instead of the usual sulfuric or p-toluenesulfonic acids. This apparatus permits determination of the total acetic acid with single distillation of 30 to 35 ml. One determination after the other can be made without cleaning. A single determination requires about 75 minutes.

Alicino (2) reports that most O-acyl groups can be determined in a manner similar to that of Clarke and Christensen by dissolving the sample in acetone instead of ethyl alcohol and hydrolyzing at room temperature for 2 hours with an excess of 0.01 N sodium hydroxide. The excess sodium hydroxide is determined by titration to a phenol red end point that must remain distinctly yellow for at least 2 minutes. A blank is run with the acetone alone.

HYDROXYL

In the group analysis of organic compounds, methods for determining the hydroxyl content have received attention. Stodola (230) was the first to reduce to a micro scale the esterification with acetic anhydride–pyridine mixture. Peterson, Hedberg, and Christensen (178), using sealed reaction tubes, applied this technique to a large number of typical alcohols and phenols. Ogg, Porter, and Willits (173) have modified their macroprocedure by using a reaction vessel with glass joints.

AMINES AND AMINO ACIDS

Plein and Dewey (180) have proposed a method for identifying organic aliphatic amines and some mixtures of amines as their diliturates. Woiwod (260) has developed a paper chromatographic method for separating some compounds containing α-amino nitrogen and then determining them by a colorimetric method.

Aqvist (7) has also developed a colorimetric method, which measures as little as 0.05 to 0.20 mg. of alanine. Virtanen (237) estimates amino acids by converting them to a volatile aldehyde and then to the acid by ninhydrin oxidation.

A paper by Konlkov (125) describes a new amino acid manometric apparatus. The Hempel pipet is eliminated, the barometric manometer is replaced by a McLood gage, no separate blanks are required, and the mercury of the manometer neve falls into the reaction vessel. The apparatus is smaller and more solid than the Van Slyke apparatus.

ALDEHYDES AND KETONES

Determination of small quantities of various ketones has been described by Sozzi (213), who separates them by precipitation with 2,4-dinitrophenylhydrazine. For a mixture of two aldehydes or two ketones, Brandstätter (23), using the glass powder method of Kofler, measures the optical refraction of the resulting hydrazones from this reaction. Genevois (62) measures the aldehydes and ketones, and identifies them by the melting points of their nitro derivatives.

ANALYSIS OF MIXTURES

The use of microchemistry has been extended, and like macroanalysis, it is now used for the quantitative measurement of materials in mixtures as well as for the ultimate analysis of pure compounds.

ALCOHOL

Stodola (229) has devised a direct method based on the sensitive iodoform test of Lustgarten, which makes possible the estimation of alcohol in small amounts of material. McNally and Coleman (151) and Chaikelis and Floersheim (32) have described volumetric oxidation methods for ethanol in as little as 0.1 ml. of sample. Henry and Kirkwood (85) oxidize the ethyl alcohol to acetic acid and determine it colorimetrically with p-hydroxybiphenyl. For mixtures of esters of methyl and ethyl alcohol, Ionescu-Matiu and Popa (106) saponify with potassium hydroxide, distill the liberated alcohol, and titrate it with potassium dichromate in the presence of methylene blue leuco base.

ORGANIC ACIDS

In the analysis of acids by micromethods, procedures have been outlined for citric acid (24, 184), and for hydrocyanic acid in blood, in plant tissue, and in solution (59, 64, 185). Formic acid is determined by the reduction of mercuric chloride (75), by colorimetric determination of formaldehydes formed from the acid (76), and by a bromine oxidation method similar to the acetyl method (3). Chromatography has been employed to determine lactic acid and succinic acids in foods (36).

SUGARS

Few recent papers on sugar analyses deal with small samples. Interest appears to be centered in the accurate accounting of minute amounts of the sugars. These papers (159, 181, 193, 236, 261) include colorimetric methods for traces of glucose, methods for total sugars with the Suff-Scharol reagents, the Bertrand and Somogyi methods, and the Hawthorne paper chromatography method for separating and estimating (82) sugar.

VITAMINS

The assay of various vitamins has been microchemical only on the basis of the small amounts of the constituents sought and not on the size of the sample. Some of these methods have been for B_1 (92, 196, 238) and for C (29, 132, 146, 186).

FATTY ACIDS

Stetten and Grail (217) have described a titration method applicable to the analysis of 8- to 20-mg. samples of fatty acids. Schmidt-Nielson (199), after saponification, isolates the fatty acid with toluene and titrates it with 0.02 N base to a thymol blue end point. Gorbach (68) describes a fat extraction microapparatus suitable for the microanalysis of fat in oilseeds. A method for estimating oleic and linoleic acids which consists in

the oxidation of the acids to dihydroxystearic and satinic acids, respectively, has been described by Gorbach and Malissa (72). The oxidation products are quantitatively separated and identified.

Spatt and Schneider (214) have used the partition coefficient of organic acids between ether and water for their identification. The amount of organic acid in the water is determined by titration.

Hill (90) has developed a colorimetric method for estimating the amount of fatty acids, esters, or methylated fatty acids by forming hydroxamic acid. A colored complex can be produced with alcoholic ferric perchlorate. For determining volatile fatty acids in blood, McClendon (150) has developed a special still for steam distillation. Distillation curves are presented for volatile acids found in blood filtrate.

SAPONIFICATION

Until recently, microchemists have been reluctant to attempt the quantitative saponification of esters, regardless of whether they exist as pure compounds or as mixtures in natural fats and oils. The need, however, has been great, for it offers an excellent method for determining molecular weight of pure material. Mitchell, Smith, and Money (158) have developed a method in which the saponification is conducted in a closed system with $2N$ sodium hydroxide. The authors specify alcohol containing 10% water, but do not discuss this important point. They do observe and discuss steric hindrance. Marcali and Rieman (154) and Ketchum (115) have proposed a method using the double indicator method of Rieman. It is applicable to decigram, centigram, and milligram samples, but they recommend the decigram sample.

One of the principal difficulties in microsaponification is to have enough alkali in the reaction mixture to ensure complete de-esterification; another is to measure the excess of alkali with the requisite accuracy. Fischer employs a method for determining acetyl groups which may be applicable to the saponification problem (15). The de-esterification is conducted with a large excess of alkali (added as $0.5N$ sodium hydroxide), which tends to reduce the time and ensure the completeness of the saponification. The large excess of alkali does not interfere, as it is removed by a hydrogen ion exchanger and the sodium salt of the organic acid is converted to the organic acid by the same treatment. The results is that the organic acid sought can be directly titrated. If it does not have a sharp end point, excess of a standard base of low titer can be added and back-titrated with a low titer acid.

DESICCATION

Barraclough (10) has presented a list of desiccants and discusses their application. Pavelka (176) calls attention to the possible moistening of the dried sample each time the desiccator is opened. To avoid this, it is recommended that the material be dried and stored in a Pregl absorption tube, which permits working with any desired gas, drying at any temperature, and using any vacuum. The drying gases can be dried with any number of desiccants, but phosphorus pentoxide was by far the best of 11 desiccants tested.

In the purification of compounds, many are separated from alcoholic solutions, and some will retain a few molecules of the alcohol, regardless of the drying conditions. A simple but little-known method, the literature reference to which is unknown, is to place such an alcoholated sample in a moist atmosphere, as in a vacuum desiccator containing an open vessel of water, for 8 to 18 hours. Water in the vapor will displace the alcohol, and the moistened sample can then be easily freed of the water by drying.

method of drying analytical samples that is becoming popular is to use as dry an atmosphere as can be obtained rather than a high vacuum. This is best accomplished by placing the sample in a drying chamber and drawing a stream of dry air or gas through the chamber.

Nash (165) has described an apparatus with which a complete analysis of 0.1 to 1 ml. of a gas sample for such constituents as carbon dioxide, carbon monoxide, methane, hydrogen, and nitrogen can be made with an accuracy of a few tenths of 1%.

Kenty and Reuter (113) have described a microapparatus with which the gases are analyzed by physical measurement except for carbon monoxide and hydrogen, which must be removed by oxidation with oxygen and a tungsten filament. The other gas components are measurd by their condensation points, comparison readings of Pirani and McLeod gages, and differences in speeds of flow. Samples consisting of only a few microliters can be analyzed with relatively high accuracy.

For analysis of mixtures of nitric oxide with hydrogen and nitrogen, Smith and Leighton (208) have given procedures whereby the nitric oxide is removed by oxygen in the presence of sodium hydroxide. Ammonia can be separated from nitrous oxide by absorption by monochloroacetic acid. LeRoy and Steacie (140) use alkaline sodium sulfate to absorb nitric oxide; unlike the previous method, this can be used in the presence of combustible gases. Instead of measuring nitric oxide in gases by the decrease in volume after absorption, Kieselbach (117) passes the gas through an alkaline permanganate solution, after which the solution is analyzed by a micro-Devarda method. An accuracy of 99% can be obtained.

For determining carbon monoxide in solution, Smaller and Hall (207) use a method based on the amount of iodine liberated in the oxidation with iodine pentoxide, which can be measured spectrophotometrically at 350 mμ. The method is reliable for concentrations of carbon monoxide as low as 0.005%, and an accuracy of 3 to 10%, depending on the carbon monoxide concentration, can be expected.

Salsbury, Cole, and Yoe (194) determine carbon monoxide gravimetrically by absorption on Ascarite after it has been oxidized to carbon dioxide by passing over Hopcalite at 195° C. Analysis of gases containing carbon monoxide in concentrations of 0.002 to 0.1% can be made. A method of analysis for carbon dioxide and oxygen in 0.7 to 0.14 cu. mm. of blood with an accuracy of ±0.6 to ±1.5% is described by Scholander and Irving (201). The micrometer buret described by Scholander and Evans (200) is used. For determining carbon monoxide in air, Beckman, McCullough, and Crane (12) have devised a portable instrument which makes use of two reactions, one between carbon monoxide and red mercuric oxide, yielding mercury vapor and carbon dioxide, and the other between mercury vapor and selenium sulfide. The latter is on a test strip, and as the air containing the mercury flows along the strip, a black coloration is produced. The method is applicable over a wide range of concentrations of carbon monoxide.

Sendroy and Granville (203) have applied their method of microanalytical reflectance spectrophotometry to the determination of carbon monoxide in air. A thin layer of palladium chloride on white paper is exposed to the air, and the change in color is measured.

Kinsey and Grant (118) have described a method for measuring mustard gas. The gas in amounts ranging from 0.5 to 200 micrograms is absorbed in organic solvents which contain chloramine-T. The residual chlorine is determined iodometrically.

TITRIMETRY

Titrimetric determinations of solutions of acetic acid by acidimetric, potentiometric, and iodometric methods have been compared by Hurka (102). The acidimetric method was satisfactory with 0.01 N sodium hydroxide, thymol blue indicator, and a final volume not to exceed 35 ml. For low concentrations of the acid, however, the iodometric procedure is highly recommended Ingold (103) recommends that the titration of organic acids be conducted potentiometrically with a glass electrode. By using the buret which he describes, which can be read to 0.0001 ml., and

performing the titration in a depression in the glass electrode, 300 to 900 micrograms of an organic acid of molecular weights from 100 to 500 can be determined.

The titrations of extremely dilute chromate solutions with ferrous iron, with a rotating platinum wire as the indicator electrode, has been described by Kolthoff and May (124). The method is said to be accurate to within 5% at concentrations of 1 to 2×10^{-4} M chromate.

Stock (223) has been able to measure the diffusion current of the reagent or of the substance under investigation by adjusting the potential of a rotating microelectrode with a potentiometer. The end point of the titration is located by the intersection of the two straight lines obtained by plotting current reading against buret reading. Some applications are the titration of halides with silver nitrate, arsenious trioxide with potassium bromate, and chromate ions with ferrous iron.

Stock (224) has also made microchemical application of the dead-stop end-point titration method, so that it can be used on the one-drop scale. The conditions must be so arranged that the solution to be titrated contains a substance able to destroy the polarization at either electrode. Stock suggests many uses and devices by which depolarization can be maintained up to the end point, such as the titration of thiosulfate ions with iodine. In his paper on microchemical applications of potentiometry (221), he gives the design of suitable apparatus for the one-drop scale titration. The rotating dish, vibrating ring, horizontal shaker, and an electrotitrimeter are used.

MOISTURE

The Karl Fischer method is not entirely suitable for determination of low moisture, especially in micro- and semimicrosamples, as the titer of the reagent must be low in order that adequate volumes of the reagent will be required, making differential readings practical. Levy, Murtaugh, and Rosenblatt (142), however, have reported satisfactory precision with the Karl Fischer reagent and a small closed system. They found that 1 to 25 mg. of water could be determined with an accuracy of ± 20 to 100 micrograms.

Lindner (144) recommends the use of naphthyl oxychlorophosphine. One gram of the reagent will measure as much as 25 to 30 mg. of water. The reaction is best carried out at 105° C. Lindner (143) has also shown that cinnamyl chloride, used by some for the microdetermination of moisture, may give high values under certain conditions, owing to the formation of hydrochloric acid by the cinnamic acid formed and the acid chloride. Gorbach and Jurinka (71) have adapted the calcium carbide method of Jakawenko to microanalysis. The acetylene formed by the moisture present is measured volumetrically.

PHYSICAL METHODS

BOILING POINT

García (61) has described a new procedure for determining the boiling point of a pure organic liquid requiring only 2 or 5 cu. mm. The apparatus, which consists of a capillary tube and a liquid heating bath of conventional design, can be operated at either normal or reduced pressures.

VAPOR PRESSURE

Natelson and Zuckerman (166) have described a simple method for determining the vapor pressure of one drop of pure liquid with an apparatus made of materials normally in the laboratory. The method is based on the principle that when a drop is partly taken up in a capillary, with some of the liquid remaining suspended from the end, and the pressure is reduced simultaneously at both ends, the meniscus in the capillary descends rapidly when the vapor pressure of the liquid is reached. Reproducibility within 0.5 mm. of mercury can easily be obtained. It is applicable only to pure liquids or pure low melting solids. Gould, Holzman, and Niemann (73) have described a method for determining vapor pressure and heat of vaporization of organic liquids on a milligram

scale. The procedure is a modification of the Emich method for determining boiling point.

MOLECULAR WEIGHT

Csokan (46) has improved the methods of Rast (187) and Pirsc (179) for determining molecular weight of organic substances. The apparatus consists of a heated metal cylinder containing a capillary tube in which the substance is melted. The tube is so illuminated that the melting can be observed with a lens. The temperature of the capillary is measured by a sensitive galvanometer connected to a thermoelement. Ketchum (114) has improved Hallett's modification of the Menzies and Wright procedure for determining molecular weight. The heating coil of the new apparatus is so arranged that it is easily cleaned and the sample container readily recovered. Nash (163) has proposed a method based on a vapor pressure comparison method in which lowering the vapor pressure produces a determinate solution of the sample. The measurements are made with isoteniscopes. The weight of sample of nonvolatile compounds can range from 2 to 8 mg., with molecular weights not to exceed 700. The sample can easily be recovered, and the choice of solvent is not limited. Wright (262) has described an isothermic distillation method for molecular weight determinations of materials soluble in alcohol. This differs from the more general methods (168) of measuring change in volume by measuring change in weight. The alcoholic solutions of the known and the unknown are contained on filter paper instead of in tubes. Accuracy within 10% of theoretical values can be expected.

Nash (164) in the design of an effusion microapparatus has added another to the long list of methods that can be employed for the determination. The apparatus used in the methods which employ rise of the boiling point, depression of the freezing point, and isothermal distillation, has been reviewed elsewhere (79). The Nash apparatus is useful for determining the molecular weight of 0.5 ml. of permanent gas or 0.5 mg. of volatile liquid.

REFRACTIVE INDEX

In addition to the various methods of determining refractive index of organic substances described by Niederl and others, a simple method of obtaining this physical measurement of an organic compound is that of Fischer and Kocher (57). A few millimeters of the sample are mixed with powdered glass of known index of refraction. The temperature of the mixture is raised until the liquid and the glass have the same index of refraction (disappearance of the Becke line). The source of light is a sodium vapor lamp. The index of refraction at 20° C. can be computed.

MISCELLANEOUS APPARATUS

The following discussion includes only pieces of microapparatus developed or improved during the past 6 years that have not been dealt with elsewhere in this review.

BURETS AND PIPETS

During the past few years, there has been considerable interest in the development of rapid, accurate, and simple means of measuring liquid volumes. Gorbach (67) devised a microburet without a stopcock which consists of a diaphragm pump an capillary tube with a detachable tip. It has a capacity of 0.2 m and an accuracy of 0.1 cu. mm. Lundbak (147) modified a Krogh pipet by employing a micrometer screw and a ground-glass piston syringe. Rieman (188) designed a new type of buret with a mercury valve. The buret operates on a horizontal position, and the tip is bent at right angles. When in operation, the tip dips below the surface of mercury covered with the solution to b titrated. The liquid in the buret is made to flow by raising o lowering the titration vessel. Stock and Fill (226) have designed a buret of 1-mm. bore which incorporates a built-in modification of the capillary break method of control of the gravity discharge.

Wyatt (263) designed a micrometer-controlled buret, a modification of the micropipet of Rasebury and Heyningen. Levvy (141) adapted the Conway microburet for the delivery of volumes up to ml., using a horizontal tube with a bore not exdeeding 3 mm. Winteringham (259) described a 1-mm. buret graduated in 0.01 ml., controlled by mercury in a bulb connected to the tube of the buret with a two-way glass stopcock.

An accurate buret capable of delivering up to 5×10^{-5} ml. of solution into a drop under a microscope has been described by Lascalzo and Benedetti-Pichler (134). The buret is operated by a leveling bulb containing mercury, and the solution titrated is contained in an open capillary. One end of the capillary is connected to a plunger, which is caused to move back and forth in the capillary to mix the solution. The first automatic buret with pressure control was reported by Llager and Sozzi (145). The U-shaped buret utilizes both gravity and surface tension to regulate the flow of the standard solution. Because of the small bore of the buret tube (0.2 to 2 mm.), it is possible to use standard solution as strong as 0.5 N and obtain values comparable with macromethods. Saunders (198) described a new type of buret made in a U with precision-bore tubing. The wide limb forms a 50-ml. buret.

Allan (4) has described a buret with mercury and plunger control with a centrifuge tube as titrating vessel. The solution in the vessel is mixed by a thin glass rod attached to the armature of an electric bell unit.

Natelson and Zuckerman (167) modified the Rehburg buret by introducing a capillary side tube having a diameter only a fraction of that of the calibrated portion. By using two stopcocks in the buret, the actual volume of standard liquid used from the large tube is measured by filling it to the original mark with solution held in the capillary side tube. Accuracy of 0.5% is possible with only 3 cu. mm. The buret of Benedetti-Pichler was improved by Lacourt, Stoffyn, and Timmermans (130) in that the volume of liquid delivered is not measured by indirect means. Shaeffer, Farrington, and Niemann (204) have designed a syringe buret suitable for general laboratory work; with this instrument 0.5 ml. can be delivered with a precision of ±0.5%, depending on the uniformity of the diameter of the syringe plunger.

Stock and Fill (228) described a simple syringe pipet suitable for mobile liquids like ether. Using a microscope stand and a syringe, Nordmann (169) attained high precision in measuring extremely small amounts of liquid. Gorbach (70) developed a micropipet for use with the microfilter rod, which is similar in shape but much smaller than the usual bulb micropipet. Because of its narrow tip, it can be connected to the filter rod with rubber tubing. It is filled by suction. Chinoy (33) reports a simple pipet for plant fluids. The plant tissue is ground in an upper chamber of the pipet, and the liberated liquid is carried into the fine stem by capillarity. Lazarow (136) has described a pipet in which not only is the liquid measured but which serves as a precipitating and centrifuging cell as well.

Lacourt and Timmermans (131) have outlined methods of titrimetric standardization of microburets. The delivered 0.1 N biiodate from the buret to be calibrated is titrated with 0.01 N thiosulfate measured in a 5 ± 0.01 ml. buret. The thiosulfate titer is checked with solid biiodate. The rate of flow in the microburet is kept constant (10 divisions per 40 seconds) to reduce the effect of drainage. This will give a measure of accuracy of 1% of the total buret volume.

Several new distilling microapparatus have been proposed. To cut down the distance from distilling flask to receiver, Tiedcke (233) used an "inside receiver." The basic design is suitable for liquids boiling between 60° and 300° C. It is important that the volume of the distilling flask and condenser tube chamber and connections be less than the volume which the sample will occupy when converted to vapor at the boiling point. Duffau (49) also uses the flask-within-a-flask design for the microdetermination of furfural formers.

A small glass center tube fractionating column described by Naragon and Lewis (162) has a fractionating section surrounded by a silvered vacuum jacket. Above this section is a distributor which distributes half the reflux from the head to the outer tube and half to the center tube.

CAPILLARY TUBES

Ma and Eder (148) have described a new weighing capillary for handling liquids in micro- and semimicroanalyses. It uses a simple filling technique. A solid expelling agent is eliminated. The tube is suitable for 2.50 mg. of sample.

A funnel for filling capillaries with solid material is described by Walker (240). The size of the tap end of the brass funnel is determined by the bore of the capillary. The powered material is caused to fall into the capillary by rubbing the funnel with a serrated surface such as the side of a pair of tweezers. A wire plunger is used if the capillary clogs.

GAS ANALYSIS APPARATUS

The Microvol of Sanderson (197), a device for measuring small volumes of condensable gases in vacuum apparatus, has been applied successfully to analysis of samples of soil atmosphere and should prove useful in gas microanalysis. The entire sample is measured directly, and the pressure-volume products are easily checked.

Kenty and Reuter (113) have described in detail a new apparatus originally designed for determining minute quantities of gas impurities occurring in vacuum tubes. The apparatus isolates the gas in a closed vacuum system. The constituents are frozen out, and identified by their condensation points where possible. Hydrogen and carbon monoxide are determined by ignition with oxygen, and the excess oxygen is removed by contact with a hot tungsten filament. Nitrogen is determined by its speed of exhaustion or by the ratio of the thermocouple gage reading to a McLeod gage reading.

A microanalyzer for extremely small gas samples of 0.4 to 1.0 cu. mm., which was used to determine the constituents in gas bubbles formed in animals decompressed to simulate high altitudes, has been described by Berg (19). The apparatus is applicable to the determination of carbon dioxide and oxygen. It consists of a 15-cm. (6-inch) capillary thermometer surrounded by a water jacket with an open bell-shaped absorption chamber blown at one end. The absorbent, saturated lithium chloride, is contained in a short piece of rubber tubing closed at one end and provided with a screw clamp.

For the qualitative analysis of gases on the micro scale, Belcher (13) reported and compared the Feigl-Krumholz apparatus and Feigl's modification of the Rassler apparatus. By modifying the Feigl-Rassler apparatus, the drop of reagent is prevented from being drawn from the absorber into the body of the apparatus.

For the collection of various atmospheric contaminants, the Engineering Unit, Division of Industrial Hygiene, National Institute of Health (53) has described an all-glass midget impinger, similar to the large one. By using standard-taper joints, the parts of the impinger can be changed without regard to the impinger orifice which was necessary in earlier models.

Whiteley (250) has designed a modified Van Slyke gas apparatus in which 0.1 ml. of blood can be analyzed for dissolved nitrogen with an accuracy of 0.002% by volume and a limit of sensitivity of 0.0027 microliter or 1.2×10^{-10} mole of gas. The apparatus is adaptable to measurement of the relatively insoluble gases, oxygen and carbon monoxide. It is not so desirable for the soluble gases such as carbon dioxide; however, a saturated salt solution and substitution of monobasic phosphate for lactic acid to liberate the gas from carbonate should give reasonable accuracy.

MELTING POINT

Most of the melting point microapparatus described in the past has been the microscope hot stage type which uses a variable tem-

perature metal block with encased thermometer. Hewitt (89) uses a shielded and stirred mercury bath instead of the metal block. The sample to be observed is placed on a cover slip which in turn is floated on the heated mercury; the melting of the sample is observed by means of a microscope. The mercury is heated either by a water bath up to 90° C. or by graphite up to 150°. The apparatus should not be used above 150° because of the volatility of the mercury. The method permits the use of small amounts of sample and fairly accurate measurements of the temperature of the solid. The probable accuracy is ±0.1° C.

MANOMETERS

To eliminate the barometric correction necessary with the instruments of Linderstrom-Lang and Boell, Rocher (190) has employed the principle of the Cartesian diver. The apparatus is suitable for the study of small quantities of substances that absorb and liberate gases. LeRoy (139) has reported a gage which measures pressure changes with an accuracy of 0.01 mm., with a total pressure of 50 mm. or more.

Brow and Schwertz (25) report a sensitive two-liquid micromanometer constructed in such a way that the meniscus between the liquids is located in a capillary section. A slight shift in the levels of the wide-end meniscus causes a great shift in the meniscus in the capillary. Young and Taylor (266) have designed a micromanometer useful in molecular weight determinations and in the field of mass spectrometry. The apparatus is a vacuum gage operating on the manometric principle; it is usable for pressure ranges of 10^{-1} to 10^{-3} mm. of mercury.

ELECTRON MICROSCOPE

The electron microscope has been little used in organic microchemistry. Gulbransen, Phelps, and Langer (78) have suggested its use in connection with the light microscope, and have predicted that it will be used mainly in the study of precipitates undetectable by the light microscope. They have proposed preparation of samples by diffusion of liquid and solids into a thin Parlodion film.

PYCNOMETERS

Houghton (97) has described a pycnometer for measuring 0.01 ml. or less of liquid. It is filled by either suction or capillary action.

Lauder (135) has published notes on factors which determine the working range of the Gilfillan-Polanyi micropycnometer. The variation of the density with the pressure of the water in which the pycnometer floats and in the body of the float is taken into consideration.

CONDUCTION CELLS

The inherent difficulty with conduction cells in microanalysis when minute amounts of liquid are used is that the electrodes are not sufficiently wetted by the solution. Blake (20) has described an apparatus in which the cell is replaced by a conductometric tube fitted with electrodes not in contact with the solution. Current is supplied from a small 1175-kc. radio frequency oscillator. The modified cell or tube is easily cleaned and can be used with colloids or precipitates.

ELECTRODES

A new glass microelectrode for pH determination has been described by Chinoy (34). This electrode consists of a vertical thick-walled tube of Corning 0.015 conductivity glass with an eccentrically blown bulb at its base. A cup-shaped conducting membrane 7 mm. deep and approximately 25 microns thick is blown in the upper region of the tube, which is filled with a saturated solution of quinhydrone in N hydrochloric acid. Contact is made by a platinum wire extending between a gold-plated terminal at the top of the tube and the liquid. Contact

between the solution of unknown pH in the cup and a saturated calomel electrode is made by a potassium chloride bridge. The cell has shown close agreement with the standard glass electrode for a variety of buffer solutions.

West and Amis (244) have described a calomel microelectrode that can be used internally with the dropping electrode for polarographic measurements. This gives results for potentials which agree closely with published values.

EXTRACTORS

Hauptmann (81) and others have made a simple microextractor by placing the sample in the longer limb of the U-tube, the top of which is funnel-shaped. The U-tube is suspended in a tube of large diameter, the bottom of which is connected to a boiling flask and the top to a condenser.

To prevent losses during extractions with small separatory funnels even when there is a favorable partition coefficient, Wayman and Wright (243) have designed continuous liquid-liquid extractors with capacities of 1 to 30 ml. Two extractors were made, one for immiscible solvents lighter than water and the other for solvents heavier than water. An apparatus for extracting organic preparation from an aqueous medium on a microscale has been described by Stock and Fill (227). Efficient separation of immiscible liquids permits the use of small volumes of the extracting liquid even when the solution being extracted is large.

A rapid microextractor for lipides with ethyl alcohol has been described by Hsiao (101). The apparatus consists of a cold finger extending into the neck of a 100-ml. Kjeldahl flask. A vial with a sintered-glass bottom contains the sample and hangs within another vial beneath the condenser. A micro filter thimble 10 mm. long and 5 mm. in diameter has been described by Bowden (22). The thimble, made of Whatman paper and closed at the bottom is placed in a cylindrical funnel and used for filtering small volumes of liquids.

MICROMANIPULATOR

Cailloux (30) describes a new micromanipulator composed of two parts—the manipulator proper and the receptor, which holds the tools. This manipulator is operated by a lever and a hydraulic system of pistons and diaphragms. As there is little loss in motion with such a system, the tools can be closely and accurately controlled.

An automatic vacuum microstirrer has been described by Fill and Stock (56). The apparatus has a stirrer plunger which rests in a pool of mercury. As vacuum is applied and released, causing the mercury to vibrate, the vibrations are transferred to the plunger rod, which in turn is connected to the vessel containing the material to be stirred.

TITRATION RACKS

As an aid in the titration of numerous samples, Cantino (31) has suggested a simple titration rack which holds eight titration (centrifuge) tubes in such a position that the tubes coincide with an aerating device. By moving the reagent bottle and attached buret from one tube to the next, a series of eight determinations can be completed rapidly.

VISCOMETERS

Two types of microviscometers have been described. The one by Weyer (245) measures the viscosity of the liquid in terms of the resistance to the torque between two parallel and horizontal disks, one driven and the other stationary. That of Glynn and Grunberg (65) measures the time required for the meniscus of the liquid to move under constant pressure from one point to another in a capillary tube placed in a horizontal position. Directions are given for calibrating the tube and for correction to be applied. When systematic variations occurred, they could be traced to thixotropic or anomalous behavior.

The literature on microchemical methods is extensive, and consequently many reviews have been prepared on various specific subjects. Some of them are listed below.

Qualitative analysis (123). A comprehensive survey of qualitative methods for the identification of organic substances, including thermal analyses, with a tabulation of 600 substances.

The history (91), development (195), and advantages of microchemical analysis (84).

Microchemical instruction (256).

Practical applications, construction, equipment, laboratory management (155) and microanalytical laboratories in America (242).

Methods and applications (14) and techniques (17).

Physicochemical methods, viscosity, surface tension, refractive index (254), and density (253).

Filtration, including centrifuging, siphoning, capillary, filter sticks, and crucibles (28, 264).

Apparatus and methods for titrimetric, gasometric, and photometric microanalysis (21). Solids purification (54) and recrystallization (45).

Microchemical quantitative analysis in clinical laboratories, history and current trends (209), and tests suitable for pharmaceutical laboratories (189).

Biological applications of microanalytical methods (18, 212).

Fat and oil analysis, new apparatus (69).

Polarographic microanalysis (222).

Automatic microchemical organic analysis (111).

Microchemistry and its borderline, and the application of x-ray, ultraviolet, visible, and infrared spectrophotometers, and electron microscope to microchemistry (157).

Submicroanalysis (255).

ACKNOWLEDGMENT

LITERATURE CITED

(1) Alicino, J. F., ANAL. CHEM., 20, 85 (1948).
(2) Ibid., 20, 590 (1948).
(3) Alicino, J. F., IND. ENG. CHEM., ANAL. ED., 15, 764 (1943).
(4) Allan, J. C., S. African J. Med. Sci., 11, 157 (1946).
(5) Aluise, V. A., Hall, R. T., Staats, F. C., and Becker, W. W., ANAL. CHEM., 19, 347 (1947).
(6) Anon., Chem. Eng. News, 26, 2580 (1948).
(7) Aqvist, S. E. G., Acta Physiol. Scand., 13, 297 (1947).
(8) Balis, E. W., Liebhafsky, H. A., and Bronk, L. B., IND. ENG. CHEM., ANAL. ED., 17, 56 (1945).
(9) Ballentine, R., and Gregg, J. R., ANAL. CHEM., 19, 281 (1947).
(10) Barraclough, K. C., Metallurgia, 31, 269 (1945).
(11) Beatty, C., III, IND. ENG. CHEM., ANAL. ED., 15, 476 (1943).
(12) Beckman, A. O., McCullough, J. D., and Crane, R. A., ANAL. CHEM., 20, 674 (1948).
(13) Belcher, R., Metallurgia, 35, 310 (1947).
(14) Belcher, R., "Microanalysis, Methods and Applications," Monograph, London Royal Institution of Chemistry, 1946; J. Textile Inst., 38, 194A (1947).
(15) Belcher, R., and Phillips, D. F., BIOS No. 1606 (British Intelligence Objectives Sub-Committee).
(16) Belcher, R., and Spooner, C. E., Ind. Chemist, 19, 653 (1943).
(17) Belcher, R., and Spoonhower, C. E., Nature, 153, 24 (1944).
(18) Benedetti-Pichler, A. A., Ann. Rev. Biochem., 12, 639 (1943).
(19) Berg, W. E., Science, 104, 575 (1946).
(20) Blake, G. G., Australian J. Sci., 10, 80 (1947).
(21) Blix, G., Iva, 1943, 382; Chem. Zentr., 1944, I, 1029.
(22) Bowden, S. T., Analyst, 72, 542 (1947).
(23) Brandstätter, M., Mikrochemie ver. Mikrochim. Acta, 32, 162 (1944).
(24) Breusch, F. L., and Tulus, R., Biochim. Biophys. Acta, 1, 77 (1947).
(25) Brow, J. E., and Schwertz, F. A., Rev. Sci. Instruments, 18, 183 (1947).
(26) Brüel, D., Holter, H., et al., Compt. rend. trav. lab. Carlsberg Ser. chim., 25, 289 (1947).
(27) Bürger, K., Die Chemie, 55, 245 (1942).
(28) Burton, F., Metallurgia, 32, 285 (1945).
(29) Butler, A. M., Cushman, M., and MacLachlan, E. A., J. Biol. Chem., 150, 453 (1943).
(30) Cailloux, M., Rev. Can. Biol., 2, 528 (1943).
(31) Cantino, E. C., IND. ENG. CHEM., ANAL. ED., 16, 346 (1944).
(32) Chaikelis, A. S., and Floersheim, R. D., Am. J. Clin. Path., Tech. Sect., 10, 180 (1946).
(33) Chinoy, J. J., Current Sci., 14, 102 (1945).
(34) Ibid., 16, 25 (1947).
(35) Christensen, B. G., Ugeskrift Laeger, 105, 866 (1943).
(36) Claborn, H. V., and Patterson, W. I., J. Assoc. Official Agr. Chem., 31, 134 (1948).
(37) Clark, E. P., J. Am. Chem. Soc., 51, 1479 (1929).
(38) Clark, E. P., J. Assoc. Official Agr. Chem., 24, 641 (1941).
(39) Clark, J. W., Rev. Sci. Instruments, 18, 915 (1947).
(40) Clark, R. O., and Stillson, G. H., ANAL. CHEM., 19, 423 (1947).
(41) Clarke, R., and Christensen, B. E., IND. ENG. CHEM., ANAL. ED., 17, 334 (1945).
(42) Cole, J. O., and Parks, C. R., Ibid., 18, 61 (1946).
(43) Corwin, A. H., Ibid., 16, 258 (1944).
(44) Corwin, A. H., paper presented at 94th meeting, AM. CHEM. Soc., Sept. 9, 1937.
(45) Craig, L. C., and Post, O. W., IND. ENG. CHEM., ANAL. ED., 16, 413 (1944).
(46) Csokan, P., Magyar Chim. Folyoirat, 48, 56 (1942).
(47) Dean, R. B., and Hawley, R. L., ANAL. CHEM., 19, 841 (1947).
(48) Dubouloz, P., Mange-Hedde, M. F., and Fonderai, J., Bull. soc. chim. France, 1947, 898.
(49) Duffau, F., Bull. Soc. Chim. Biol., 28, 873 (1946).
(50) Elving, P. J., and Ligett, W. B., Chem. Revs., 34, 129 (1944).
(51) Elving, P. J., and Ligett, W. B., IND. ENG. CHEM., ANAL. ED., 14, 449 (1942).
(52) Elving, P. J., and McElroy, W. R., Ibid., 13, 660 (1941).
(53) Engineering Unit, Div. Industrial Hygiene, Natl. Inst. Health, Ibid., 16, 346 (1944).
(54) English, James, Jr., Ibid., 16, 478 (1944).
(55) Etienne, A., and Mileur, R., Ann. chim. anal., 28, 215 (1946).
(56) Fill, M. A., and Stock, J. T., Analyst, 69, 212 (1944).
(57) Fischer, R., and Kocher, G., Mikrochemie ver. Mikrochim. Acta, 32, 173 (1944).
(58) Freri, M., Gass. chim. ital., 76, 108 (1946).
(59) Fulton, R. A., and Van Dyke, M. J., ANAL. CHEM., 19, 922 (1947).
(60) Furter, M. F., and Steyermark, Al, Ibid., 20, 257 (1948).
(61) García, C. R., IND. ENG. CHEM., ANAL. ED., 15, 648 (1943).
(62) Genevois, L., Chim. anal., 29, 77 (1947).
(63) Ibid., 29, 101 (1947).
(64) Gettler, A. O., and Goldbaum, L., ANAL. CHEM., 19, 270 (1947).
(65) Glynn, E., and Grunberg, L., J. Inst. Petroleum, 34, 331 (1948).
(66) Gonick, H., Tunnicliff, D. D., Peters, E. D., Lykken, L., and Zahn, V., IND. ENG. CHEM., ANAL. ED., 17, 677 (1945).
(67) Gorbach, G., Chem. Fabrik, 14, 390 (1941).
(68) Gorbach, G., Fette u. Seifen, 49, 625 (1942).
(69) Ibid., 51, 6 (1944).
(70) Gorbach, G., Mikrochemie ver. Mikrochim. Acta, 31, 109 (1943).
(71) Gorbach, G., and Jurinka, A., Fette u. Seifen, 51, 129 (1944).
(72) Gorbach, G., and Malissa, H., Mikrochemie ver. Mikrochim. Acta, 33, 145 (1947).
(73) Gould, C., Jr., Holzman, G., and Niemann, C., ANAL. CHEM., 19, 204 (1947).
(74) Goulden, F., Analyst, 73, 320 (1948).
(75) Grant, W. M., ANAL. CHEM., 19, 206 (1947).
(76) Ibid., 20, 267 (1948).
(77) Gulbransen, E. A., Rev. Sci. Instruments, 15, 201 (1944).
(78) Gulbransen, E. A., Phelps, R. T., and Langer, A., IND. ENG. CHEM., ANAL. ED., 17, 646 (1945).
(79) Hallett, L. T., Ibid., 14, 956 (1942).
(80) Harlay, V., Ann. pharm. franç., 5, 81 (1947).
(81) Hauptmann, H., and Franca, J., Anais assoc. quim. Brasil, 2, 126 (1943).
(82) Hawthorne, J. R., Nature, 160, 714 (1947).
(83) Heinemann, Heinz, Oil and Soap, 23, 227 (1946).
(84) Hel'man, N. E., and Korshun, M. O., Trudy Vsesoyuz Konferentsii Anal. Khim., Akad. Nauk. U.S.S.R., 3, 36 (1944).
(85) Henry, R. J., Kirkwood, C. F., et al., J. Lab. Clin. Med., 33, 241 (1948).
(86) Heron, A. E., Analyst, 72, 142 (1947).
(87) Ibid., 73, 314 (1948).
(88) Hershberg, E. B., and Southworth, L., ANAL. CHEM., 11, 404 (1939).
(89) Hewitt, E. J., Chemistry & Industry, 1947, 42.
(90) Hill, U. T., IND. ENG. CHEM., ANAL. ED., 18, 317 (1946).
(91) Hillis, M. O., J. Chem. Education, 22, 348 (1945).
(92) Hinton, J. J. C., Biochem. J., 37, 585 (1943).
(93) Hoffman, D. O., and Wolfrom, M. L., ANAL. CHEM., 19, 225 (1947).
(94) Horeischy, K., and Bühler, F., Mikrochemie ver. Mikrochim. Acta, 33, 231 (1947).
(95) Horeischy, Kurt, Dreher, I., and Hoffmann-Ostenhof, O., Ibid., 33, 221 (1948).
(96) Horning, E. C., and Horning, M. G., ANAL. CHEM., 19, 688 (1947).
(97) Houghton, A. A., Analyst, 69, 345 (1944).

(98) *Ibid.*, 70, 19 (1945).
(99) *Ibid.*, 70, 118 (1945).
(100) Houghton, A. A., and Wilson, H. A. B., *Ibid.*, 69, 363 (1944).
(101) Hsiao, S. C., *Science*, 107, 24–5 (1948).
(102) Hurka, W., *Mikrochemie ver. Mikrochim. Acta*, 31, 5 (1943).
(103) Ingold, W., *Helv. Chim. Acta*, 29, 1929 (1946).
(104) Ingram, G., *Analyst*, 69, 265 (1944).
(105) Ingram, G., *J. Soc. Chem. Ind.*, 62, 175 (1943).
(106) Ionescu-Matiu, A., and Popa, I., *Products Pharm.*, 2, 389 (1947).
(107) Irimescu, I., and Chirnoaga, E., *Z. anal. Chem.*, 125, 32 (1942).
(108) *Ibid.*, 128, 71 (1947).
(109) Irimescu, I., and Popescu, B., *Ibid.*, 128, 185 (1948).
(110) Jurecek, M., *Collection Czech. Chem. Communs.*, 12, 455 (1947).
(111) Kahane, E., *Chem. anal.*, 30, 100 (1948).
(112) Kaye, I. A., and Weiner, N., IND. ENG. CHEM., ANAL. ED., 17, 397 (1945).
(113) Kenty, C., and Reuter, F. W., Jr., *Rev. Sci. Instruments*, 18, 918 (1947).
(114) Ketchum, D., ANAL. CHEM., 19, 504 (1947).
(115) Ketchum, D., IND. ENG. CHEM., ANAL. ED., 18, 273 (1946).
(116) Kieselbach, R., *Ibid.*, 16, 764 (1944).
(117) *Ibid.*, 16, 766 (1944).
(118) Kinsey, V. E., and Grant, W. M., *Ibid.*, 18, 794 (1946).
(119) Kirk, Paul L., Symposium on Kjeldahl Nitrogen, 114th Meeting of AM. CHEM. SOC., St. Louis, Mo., 1948.
(120) Kirk, P. L., Craig, R., Gullberg, J. E., and Boyer, R. Q., ANAL. CHEM., 19, 427 (1947).
(121) Kirsten, Wolfgang, *Ibid.*, 19, 925 (1947).
(122) Klein, B., IND. ENG. CHEM., ANAL. ED., 16, 536 (1944).
(123) Kofler, L., *Beih. Z. ver deut. Chem., A, Chem., B, Chem. Tech.*, 1942, No. 46, 1.
(124) Kolthoff, I. M., and May, D. R., IND. ENG. CHEM., ANAL. ED., 18, 208 (1946).
(125) Konikov, A. P., *Biokhimiya*, 12, 221 (1947).
(126) Korshun, M. O., and Hel'man, N. E., *Zavodskaya Lab.*, 12, 500 (1946).
(127) *Ibid.*, 12, 617 (1946).
(128) *Ibid.*, 12, 754 (1946).
(129) Lacourt, A., *Metallurgia*, 36, 289 (1947).
(130) Lacourt, A., Stoffyn, P., and Timmermans, A. M., *Mikrochemie ver. Mikrochim. Acta*, 33, 217 (1947).
(131) Lacourt, A., and Timmermans, A. M., *Bull. classe sci. Acad. roy. Belg.*, 32, 52 (1946).
(132) Lamb, F. W., IND. ENG. CHEM., ANAL. ED., 15, 352 (1943).
(133) Langer, A., *Ibid.*, 17, 266 (1945).
(134) Lascalzo, A. G., and Benedetti-Pichler, A. A., *Ibid.*, 17, 187 (1945).
(135) Lauder, I., *Trans. Faraday Soc.*, 44, 78 (1948).
(136) Lazarow, A., *J. Lab. Clin. Med.*, 32, 213 (1947).
(137) Leithe, W., *Mikrochemie ver. Mikrochim. Acta*, 33, 149 (1947).
(138) *Ibid.*, 33, 167 (1947).
(139) LeRoy, D. J., IND. ENG. CHEM., ANAL. ED., 17, 652 (1945).
(140) LeRoy, D. J., and Steacie, E. W. R., *Ibid.*, 16, 341 (1944).
(141) Levvy, G. A., *Chemistry & Industry*, 1945, 4.
(142) Levy, G. B., Murtaugh, J. J., and Rosenblatt, M., IND. ENG. CHEM., ANAL. ED., 17, 193 (1945).
(143) Lindner, J., *Mikrochemie ver. Mikrochim. Acta*, 32, 133 (1944).
(144) *Ibid.*, 32, 155 (1944).
(145) Llager, A. J., and Sozzi, J. A., *Anales farm. y bioquim.*, 16, 82 (1945).
(146) Lucas, E. H., IND. ENG. CHEM., ANAL. ED., 16, 649 (1944).
(147) Lundbak, A., *Kem. Maanedsblad*, 24, 138 (1943).
(148) Ma, T. S., and Eder, K. W., *J. Chinese Chem. Soc.*, 15, 112 (1947).
(149) Ma, T. S., and Zuazaga, G., IND. ENG. CHEM., ANAL. ED., 14, 280 (1942).
(150) McClendon, J. F., *J. Biol. Chem.*, 154, 357–60 (1944).
(151) McNally, W. D., and Coleman, H. M., *J. Lab. Clin. Med.*, 29, 429 (1944).
(152) MacNevin, W. M., and Varner, J. E., IND. ENG. CHEM., ANAL. ED., 15, 224 (1943).
(153) Manigault, P., and Tsai, B., *Compt. rend.*, 214, 658 (1942).
(154) Marcali, K., and Rieman, Wm., III, IND. ENG. CHEM., ANAL. ED., 18, 144 (1946).
(155) Martin, F., *Ann. chim. anal.*, 28, 219 (1946).
(156) Martin, F., *Chim. anal.*, 30, 4–8, 37–40 (1948).
(157) Masters, D. L., *Metallurgia*, 30, 169 (1944).
(158) Mitchell, J., Jr., Smith, D. M., and Money, F. S., IND. ENG. CHEM., ANAL. ED., 16, 410 (1944).
(159) Morris, Daniel L., *J. Biol. Chem.*, 154, 561 (1944).
(160) Müller, A., *Mikrochemie ver. Mikrochim. Acta*, 33, 192 (1947).
(161) Nanjundayya, C., and Ahmad, N., *Indian J. Agr. Sci.*, 13, 649 (1943).
(162) Naragon, E. A., and Lewis, C. J., IND. ENG. CHEM., ANAL. ED., 18, 448 (1946).

(163) Nash, L. K., ANAL. CHEM., 19, 799 (1947).
(164) *Ibid.*, 20, 258 (1948).
(165) Nash, L. K., IND. ENG. CHEM., ANAL. ED., 18, 505 (1946).
(166) Natelson, S., and Zuckerman, J. L., *Ibid.*, 17, 739 (1945).
(167) Natelson, S., and Zuckerman, J. L., *J. Biol. Chem.*, 170, 305 (1947).
(168) Niederl and Niederl, "Micromethods of Quantitative Organic Analysis," 2nd ed., p. 230, New York, John Wiley & Son, 1942.
(169) Nordmann, J., *Ann. biol. clin. (Paris)*, 5, 401 (1947).
(170) Northrop, J. H., *J. Gen. Physiol.*, 31, 213 (1948).
(171) Oberháuser, B. F., Rivera, R. A., and Etcheverry, D. H., *Anales facultad filosof. y educación, Univ. Chile, Sección quim*, 3, 61 (1946).
(172) Ogg, C. L., Brand, R. W., and Willits, C. O., *J. Assoc. Official Agr. Chem.*, 31, 663 (1948).
(173) Ogg, C. L., Porter, W. L., and Willits, C. O., IND. ENG. CHEM., ANAL. ED., 17, 394 (1945).
(174) Ogg, C. L., Willits, C. O., and Cooper, F. J., ANAL. CHEM., 20, 83 (1948).
(175) Pagel, H. A., IND. ENG. CHEM., ANAL. ED., 16, 344 (1944).
(176) Pavelka, F., *Mikrochemie ver. Mikrochim. Acta*, 32, 141 (1944).
(177) Peel, E. W., Clark, R. H., and Wagner, E. C., IND. ENG. CHEM., ANAL. ED., 15, 149 (1943).
(178) Petersen, J. W., Hedberg, K. W., and Christensen, B. E., *Ibid.*, 15, 225 (1943).
(179) Pirsch, J., *Angew. Chem.*, 51, 73 (1938).
(180) Plein, E. M., and Dewey, B. T., IND. ENG. CHEM., ANAL. ED., 15, 534 (1943).
(181) Polis, B. D., and Sortwell, M., *Arch. Biochem.*, 11, 229 (1946).
(182) Pomatti, R., IND. ENG. CHEM., ANAL. ED., 18, 63 (1946).
(183) Portner, P. E., ANAL. CHEM., 19, 502 (1947).
(184) Pucher, G. W., *J. Biol. Chem.*, 153, 133 (1944).
(185) Quesada, R., and Moia, B., *Rev. asoc. bioquim. argentina*, 9, No. 31, 39 (1943).
(186) Raoul, Y., *Bull. soc. chim. biol.*, 29, 728 (1947).
(187) Rast, K., *Ber.*, 55B, 3727 (1922).
(188) Rieman, W., III, IND. ENG. CHEM., ANAL. ED., 16, 475 (1944).
(189) Riemers, F., *Dansk. Tids. Farm.*, 21, 1 (1947).
(190) Rocher, H., *Bull. soc. chim.*, 10, 486 (1943).
(191) Rodden, C. J., Kuck, J. A., Benedetti-Pichler, A. A., *et al.*, IND. ENG. CHEM., ANAL. ED., 15, 415 (1943).
(192) Saccardi, P., *Ann. chim. applicata*, 34, 18 (1944).
(193) Saint-Rat., L. de, and Corvazier, L., *Ann. pharm. franç.*, 4, 47 (1946).
(194) Salsbury, J. M., Cole, J. W., and Yoe, J. H., ANAL. CHEM., 19, 66 (1947).
(195) Sanborn, G., *Interchem. Rev.*, 4, 20 (1945).
(196) Sánchez, J. A., *Rev. asoc. bioquim. argentina*, 14, 35 (1947).
(197) Sanderson, R. T., IND. ENG. CHEM., ANAL. ED., 15, 76 (1943).
(198) Saunders, J. A., *Analyst*, 71, 528 (1946).
(199) Schmidt-Nielson, K., *Compt. rend. trav. lab. Carlsberg Ser. chim.*, 24, 233 (1942).
(200) Scholander, P. F., and Evans, H. J., *J. Biol. Chem.*, 169, 551 (1947).
(201) Scholander, P. F., and Irving, L., *Ibid.*, 169, 561 (1947).
(202) Schütze, M., *Z. anal. Chem.*, 118, 241 (1939).
(203) Sendroy, J., Jr., and Granville, W. C., ANAL. CHEM., 19, 500 (1947).
(204) Shaeffer, P. A., Jr., Farrington, P. S., and Niemann, C., *Ibid.*, 19, 492 (1947).
(205) Shahrokh, B. K., *J. Biol. Chem.*, 154, 517 (1944).
(206) Sisido, K., and Yagi, H., ANAL. CHEM., 20, 677 (1948).
(207) Smaller, B., and Hall, J. F., Jr., IND. ENG. CHEM., ANAL. ED., 16, 64 (1944).
(208) Smith, R. N., and Leighton, P. A., *Ibid.*, 14, 758 (1942).
(209) Sobel, A. E., *Ibid.*, 17, 242 (1945).
(210) Sobel, A. E., Hirschman, A., and Besman, L., ANAL. CHEM., 19, 927 (1947).
(211) Sobel, A. E., Mayer, A. M., and Gottfried, S. P., *J. Biol. Chem.*, 156, 355 (1944).
(212) Sozzi, J. A., *Anales farm. bioquim. Supl.*, 14, 21 (1943).
(213) Sozzi, J. A., *Anales farm. bioquim.*, 14, 41 (1943).
(214) Spatt, C., and Schneider, F., IND. ENG. CHEM., ANAL. ED., 16, 479 (1944).
(215) Spooner, C. E., *Metallurgia*, 37, 157 (1948).
(216) Stehr, E., IND. ENG. CHEM., ANAL. ED., 18, 513 (1946).
(217) Stetten, DeWitt, Jr., and Grail, G. F., *Ibid.*, 15, 300 (1943).
(218) Steyermark, Al, ANAL. CHEM., 20, 368 (1948).
(219) Steyermark, Al, IND. ENG. CHEM., ANAL. ED., 17, 523 (1945)
(220) Steyermark, Al, Bass, E., and Littman, B., ANAL. CHEM., 20, 587 (1948).
(221) Stock, J. T., *Analyst*, 73, 321 (1948).
(222) Stock, J. T., *Chem. Products*, 7, No. 3–4, 23 (1944).
(223) Stock, J. T., *Metallurgia*, 36, 51 (1947).
(224) *Ibid.*, 37, 220 (1948).

(225) Stock, J. T., and Fill, M. A., *Analyst*, 72, 394 (1947).
(226) Stock, J. T., and Fill, M. A., *Metallurgia*, 31, 103 (1944).
(227) *Ibid.*, 33, 44 (1945).
(228) *Ibid.*, 36, 225 (1947).
(229) Stodola, F. H., IND. ENG. CHEM., ANAL. ED., 15, 72 (1943).
(230) Stodola, F. H., *Mikrochemie*, 21, 180 (1937).
(231) Sundberg, O. E., and Royer, G. L., IND. ENG. CHEM., ANAL. ED., 18, 719 (1946).
(232) Teston, R. O'D., and McKenna, F. E., ANAL. CHEM., 19, 193 (1947).
(233) Tiedcke, C., IND. ENG. CHEM.; ANAL. ED., 15, 81 (1943).
(234) Tuttle, C., and Brown, F. M., *Ibid.*, 16, 645 (1944).
(235) Unterzaucher, J., *Ber.*, 73, 391 (1940).
(236) Van de Kramer, J. H., *Chem. Weekblad*, 39, 585 (1942).
(237) Virtanen, A. I., and Rautanen, N., *Suomen Kemistilehti*, 19B, 56 (1946).
(238) Wachsmuth, H., *Bull. soc. chim. belges*, 56, 261 (1947).
(239) Wagner, E. C., and Miles, S. H., ANAL. CHEM., 19, 274 (1947).
(240) Walker, A. O., IND. ENG. CHEM., ANAL. ED., 16, 343 (1944).
(241) Walton, W. W., McCulloch, F. W., and Smith, W. H., *J. Research Natl. Bur. Standards*, 40, 443 (1948).
(242) Warth, A. H., *Crown*, 34, 13 (1945).
(243) Wayman, M., and Wright, G. F., IND. ENG. CHEM., ANAL. ED., 17, 55 (1945).
(244) West, P. W., and Amis, E. S., *Science*, 101, 71 (1945).
(245) Weyer, E. R., *Ibid.*, 101, 521 (1945).
(246) Weygand, C., *Chem. Tech.*, 16, 15 (1943).
(247) White, E. P., IND. ENG. CHEM., ANAL. ED., 16, 207 (1944).
(248) White, L. M., and Secor, G. E., *Ibid.*, 18, 457 (1946).

(249) White, T., *Analyst*, 68, 366 (1943).
(250) Whiteley, A. H., *J. Biol. Chem.*, 174, 947 (1948).
(251) Wiesenberger, E., *Mikrochemie ver. Microchim. Acta*, 30, 241 (1942).
(252) *Ibid.*, 33, 51 (1947).
(253) Willits, C. O., and Ogg, C. L., *J. Assoc. Official Agr. Chem.*, in press.
(254) Wilson, C. L., *Analyst*, 71, 117 (1946).
(255) Wilson, C. L., *Chem. Age*, 56, 505 (1947).
(256) Wilson, C. L., *Chemistry & Industry*, 1945, 258.
(257) Wilson, C. L., *Metallurgia*, 31, 101 (1944).
(258) *Ibid.*, 33, 157 (1946).
(259) Winteringham, F. P. W., *Analyst*, 70, 173 (1945).
(260) Woiwod, A. J., *Nature*, 161, 169 (1948).
(261) Wolff, R., and de Lavergne, E., *Compt. rend. soc. biol.*, 141, 926 (1947).
(262) Wright, Robert, *Analyst*, 73, 387 (1948).
(263) Wyatt, G. H., *Ibid.*, 69, 180 (1944).
(264) *Ibid.*, 71, 122 (1946).
(265) Yagi, Y., and Egami, F., *J. Chem. Soc. Japan*, 67, 20 (1946).
(266) Young, W. S., and Taylor, R. C., ANAL. CHEM., 19, 133 (1947).
(267) Zacherl, M. K., and Krainick, H. G., *Mikrochemie.*, 11, 61 (1932).
(268) Zimmermann, W., *Mikrochemie ver. Mikrochim. Acta*, 31, 15 (1943).
(269) *Ibid.*, 31, 42 (1943).
(270) *Ibid.*, 33, 122 (1947).
(271) Zimmermann, W., *Z. anal. Chem.*, 118, 258 (1939).